

**APPENDIX A**

This is an interim report summarizing how each *Eimeria* species in a four-species live coccidiosis vaccine produces a protective immune response by 30 days post vaccination in chickens administered with the vaccine.

**Summary of results**

A. The data on the pooled *Eimeria* species and the individual *Eimeria* species demonstrate that post vaccination with each of the three modes of administration tested, a protective immune response to each species in the vaccine develops.

B. Mean histological lesion scores for each section of intestine examined show that when vaccinated chickens were challenged with a x80 vaccine dose at 30 days post vaccination, scores for all lesions in each section of intestine were lower in vaccinated groups compared to the control group (Tables I-IV). This is evidence that a protective immune response has developed in the vaccinated chickens, regardless of the means of administering the vaccine selected.

**TABLE I**

Treatment	Duodenum	Mid-small intestine	Mid-intestine	Mid-large intestine	Caecum	Large intestine adjacent to caecum
Control	92	82	54	62	70	8
Spray	0	37	6	7	22	0
Eye-drop	0	12	7	0	2	0
Spray + eye-drop	2	15	15	5	0	2

A summary of the mean histological lesion scores for all fields and species expressed as the percentage of field positive.

C. The pooled data was then broken up into species. Each species in the four-species vaccine can be determined based on the histopathology. The relative comparison of data is more complex due to the fact that each species of *Eimeria* has a site-specific predilection for growing.

Coccidiosis is a self-limiting disease. The lower number of lesions is a direct indication of a protective immune response. The data presented demonstrate that all species can produce a protective immune response, and the presence of the other three species did not appear to inhibit the immune response. Differences between the methods of administration is thought to be due to the differing application methods. The less accurate the method of administration, the more likely species with lower reproductive index (oocyst output per number of oocysts applied per dose) will have lower rates of immune response.

#### **Summary of trial method**

One day-old commercial broiler cockerels were vaccinated with one of three vaccination schedules:

- a. spray at one dose per chicken
- b. spray at one dose per chicken and eye-drop at 15%
- c. eye-drop only at day-old.

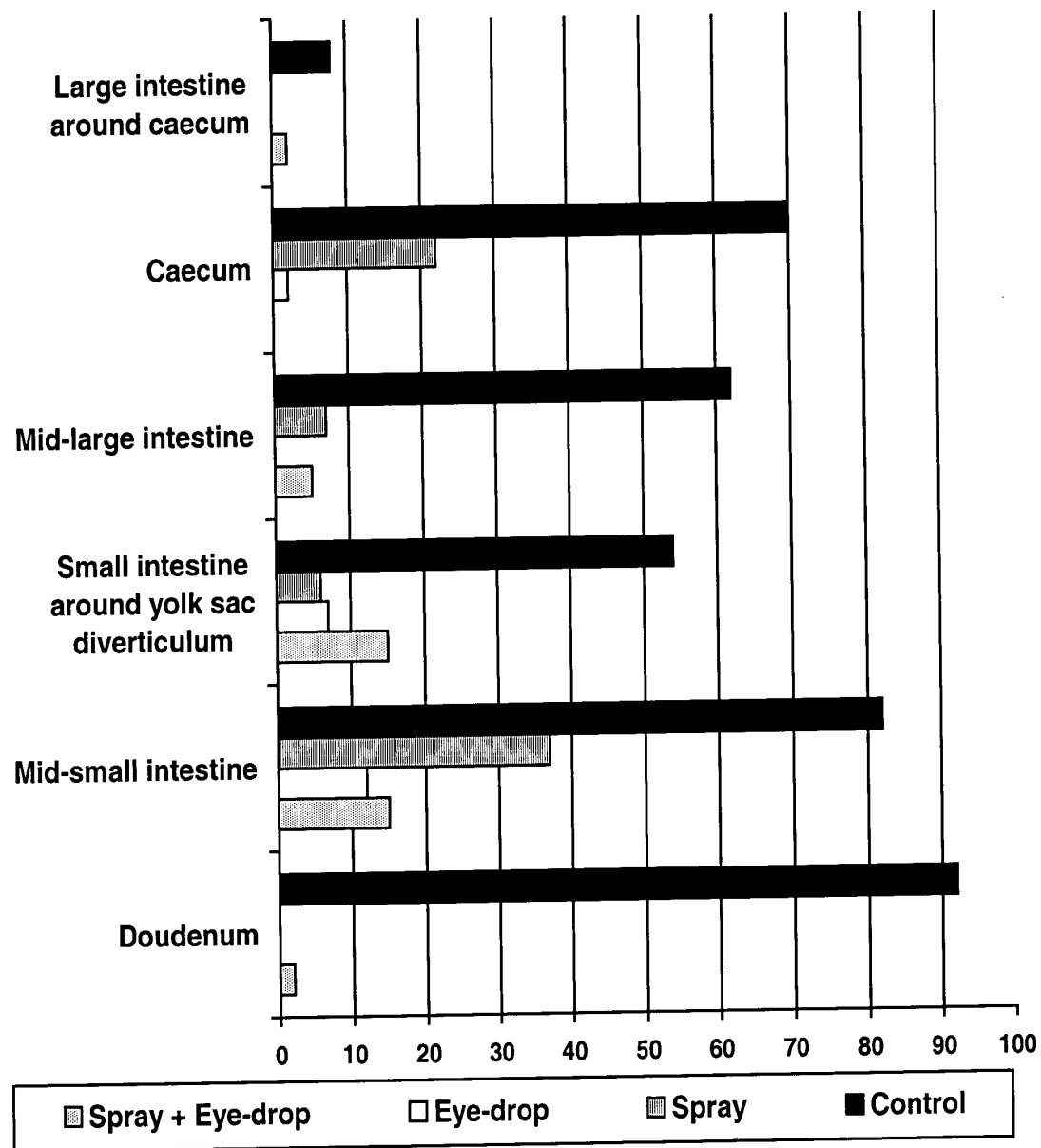
One group of chickens was raised on in-feed coccidiostat (Elancoban) at commercial rates (positive controls).

Chicken groups were reared in isolation from each other and all were housed on litter in the same environment.

At 28 days of age, six chickens from each group were removed to cages and placed onto coccidiostat free feed.

At 30 days of age, all chickens were challenged with a x80 dose of vaccine.

At 37 days of age intestinal sections were removed, fixed, stained and microscopic histological lesion enumeration was conducted.

**TABLE II: Mean histological lesion score seven days post challenge at 30 days of age**

**TABLE III: Summary of the histological data (pooled results)**

Treatment group	Doudenum	Mid-small intestine	Small intestine around yolk sac diverticulum	Mid-large intestine	Caecum	Large intestine around caecum
<b>Spray + Eye-drop</b>						
913-1	0	0	0	0	0	0
913-2	0	1	0	1	0	0
913-3	0	0	1	0	0	0
913-4	0	6	5	2	0	1
913-5	1	2	2	0	0	0
913-6	0	0	1	0	0	0
Total fields positive	1	9	9	3	0	1
Total fields examined	60	60	60	60	60	60
% positive	2%	15%	15%	5%	0%	2%
<b>Eye-drop</b>						
914-1	0	1	2	0	0	0
914-2	0	3	1	0	0	0
914-3	0	0	0	0	0	0
914-4	0	1	0	0	1	0
914-5	0	0	1	0	0	0
914-6	0	2	0	0	0	0
Total fields positive	0	7	4	0	1	0
Total fields examined	60	60	60	60	60	60
% positive	0%	12%	7%	0%	2%	0%
<b>Spray</b>						
916-1	0	2	0	0	1	0
916-2	0	4	0	1	2	0
916-3	0	4	1	1	10	0
916-4	0	0	0	0	0	0
916-5	0	4	2	2	0	0
916-6	0	8	0	0	0	0
Total fields positive	0	22	3	4	13	0
Total fields examined	60	60	50	60	60	60
% positive	0%	37%	6%	7%	22%	0%
<b>Control</b>						
915-1	7	6	6	1	10	1
915-2	10	7	7	1	8	0
915-3	10	8	10	9	1	0
915-4	12	10	0	10	6	0
915-5	7	10	4	10	10	3
Total fields positive	46	41	27	31	35	4
Total fields examined	50	50	50	50	50	50
% positive	92%	82%	54%	62%	70%	8%

**TABLE IV: Areas by species: The percentage of lesions specific for each *Eimeria* species by location**

Treatment group	Doudenum	Mid-small intestine	Small intestine around yolk sac diverticulum	Mid-large intestine	Caecum	Large intestine around caecum
<b><i>Eimeria</i> strain A</b>						
Spray + eye-drop	0	0	0	0	0	0
Eye-drop	0	0	0	0	0	0
Spray	0	0	0	0	0	0
Control	64	2	0	0	0	0
<b><i>Eimeria</i> strain B</b>						
Spray + eye-drop	6	15	15	3	0	1.6
Eye-drop	0	11.6	6.6	0	0	0
Spray	0	36.6	16	6.6	0	0
Control	0	86	12	2	0	2
<b><i>Eimeria</i> strain C</b>						
Spray + eye-drop	0	0	0	0	0	0
Eye-drop	0	0	0	0	0	0
Spray	0	0	1.6	0	0	0
Control	28	40	60	60	0	6
<b><i>Eimeria</i> strain D</b>						
Spray + eye-drop	0	0	0	0	0	0
Eye-drop	0	0	0	0	1.6	0
Spray	0	0	0	0	21.6	0
Control	0	0	0	0	70	0